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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Liew, C.C.	Examiner: Juliet C. Switzer
Serial No.: 10/268,730	
Filed: Oct. 9, 2002	Group Art Unit: 1634
Titled: Method for the Detection of Gene Transcripts in Blood and Uses Thereof	Conf. No.: 5174

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8a

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Supplemental Declaration under U.S.C. 1.132 to the Declaration dated February 28, 2006

I, Choong-Chin (C.C.) Liew, a citizen of 81 Millersgrove Drive, Toronto, Ontario, Canada M2R 3S1, declare the following:

1. I am the inventor noted on the instant application.
2. I have read and reviewed the 112 first paragraph enablement rejection applied in the Office Action dated August 28, 2006, which takes into account the Declaration sworn in this matter dated February 28, 2006 (the "Declaration of February 2006") which was filed to address similar issues raised by the examiner regarding the enablement of the invention in the Office Action dated January 4, 2006.
3. I understand the Examiner continues to apply the enablement rejection on the basis that, in her view, the Declaration of February 2006 is not commensurate in scope with the claimed subject matter. More specifically I understand that that Examiner is concerned that there is no evidence provided that the comparison of one individual's level of gene expression as against another individual's level of gene expression will be sufficient to conclude that a difference in expression is "indicative of disease".

4. Presented in the above referenced patent application are working examples teaching a method of detecting a difference in expression of two or more genes by detecting and quantifying a level of RNA encoded by each gene in an unfractionated sample of lysed blood from a test subject as compared with a quantified level of control RNA encoded by each gene in RNA of unfractionated samples of lysed blood from **one or more control subjects**, wherein the difference for each gene is indicative of disease and each gene is one expressed in blood and a non blood tissue.
5. Included herein is additional data for two genes selected from the data noted in Example 1A of the Declaration of February 2006 which are indicative of colorectal cancer; five genes selected from the data noted in Example 2A of the Declaration of February 2006 which are indicative of coronary artery disease and five genes selected from the data noted in Example 7A of the Declaration of February 2006 which are indicative of schizophrenia. For each of these genes, I have provided evidence that the comparison of one individual's level of gene expression as compared against a level of gene expression of one or more controls is sufficient to conclude that a difference in expression is indicative of disease.
6. The steps used to generate the data consisted of the following
 - (a) Ten mls of peripheral whole blood were collected in Vacutainer tubes containing EDTA (Becton Dickinson, Franklin Lakes, N.J.). Patients and controls were recruited from various Institutions and each patient was diagnosed by a registered physician.
 - (b) Total RNA was isolated from unfractionated whole blood by first treating the blood with lysis buffer (1.6 mM EDTA, 10 mM KHCO₃, 153 mM NH₄Cl, pH 7.4) and applying 1.0 ml of TRIzol[®] Reagent (Invitrogen Corp., Carlsbad, CA) and 0.2 ml of chloroform to the resulting pellet in accordance to the manufacture's instructions yielding approximately 20-30ug of RNA.
 - (c) For each individual tested, five µg of the total RNA sample isolated was used for hybridization onto the probes of predetermined sequence on the Affymetrix[®] U133Plus2 platform (Affymetrix, Santa Clara, CA) following the manufacturer's instructions. The presence of each gene for a given sample was determined by the GeneChip Operating System (GCOS) software (Affymetrix, Santa Clara, CA) which provides a **detection p value** indicative of the likelihood that the gene was present or not. Probe sets identified as absent were not utilized for further analysis. RNA was hybridized with the Affymetrix[®] GeneChip in accordance with the provided protocol. Briefly RNA samples were reverse-transcribed to cDNA using an T7 oligodT primer. Following second strand synthesis, labeled RNA is synthesized using the Enzo[®] BioArray High Yield RNA labeling kit using biotinylated nucleotides. Resulting labeled RNA is fragmented prior to hybridization. To allow comparison amongst samples, signal intensities were normalized using the GeneSpring v.6.0 software and differentially expressed genes identified by applying known statistical tests (as specifically noted below for each example). Genes were identified which differentiated as between disease and control samples at a set p value. Results were then visualized and agglomerative hierarchical cluster analysis performed using GeneSpring v 6.0 software.

7. For each of the three selected diseases discussed below, the data is presented in two parts (Part A, and Part B). As can be seen in the analysis presented below, it is possible to compare a test subject as compared with either one, or more control subjects, so as to determine a difference which is indicative of disease. In the case where a single control subject is utilized (Example 1B) a control subject was chosen which most closely corresponds to the 75th percentile of the population of the control subjects. It was well appreciated at the time of filing of the above referenced patent application that in choosing a single "control" level, the control subject should be chosen which exhibits a close correlation to standard of the population.
8. **Part A** demonstrates the application of the common statistical method of applying a box-and-whisker plot to the quantified level of gene expression for each individual in the control population (noted in green) and each individual in the disease population (noted in red) where the y axis notes the quantified level of RNA hybridized using the Affymetrix® U133 Plus 2.0 platform. The notched box-and-whisker plot shows the distribution of the data within the population including the mean (centre of the notch), the 5th percentile and 95th percentile of the distribution (end of each whisker noted in either green or red) and the 25th percentile and 75th percentile of the distribution (end of each box).

Furthermore in a notched box-and-whisker plot, the confidence intervals for the median are provided by means of notches surrounding the medians (McGill et al., 1978). If the notches about the two medians do not overlap, then the medians are considered significantly different at a $\pm 95\%$ confidence level. Also shown are bars (in blue) which represent 50% of the population surrounding the median.

Conclusion: Each box-and-whisker plot shows the distribution of gene expression levels as between disease samples and control samples for a given gene. In all cases there is a statistically significant difference as between the diseased and control populations at a $\pm 95\%$ confidence level.

9. That **Part B** is an analysis to demonstrate the utility of the application of the claimed method to measuring the difference as between a test subject and one or more control subjects. From the previous graphs noted in Part A, a threshold which differentiates as between the diseased and control subjects was determined. The threshold was chosen such that at least 75 percent of the control subjects falls above (or below) the threshold. The quantified level of RNA hybridized using the Affymetrix® U133 Plus 2.0 platform for each test subject was compared with the control subjects (not having disease) and a determination made whether the test subject demonstrated a quantified level of RNA which was greater than the 25th percentile of the control subjects. If the quantified level of RNA of the test subject was greater than the 25th percentile, the test subject was considered to be a control; if the quantified level of RNA of the test subject fell below the 25th percentile of the control subjects, the test subject was considered to demonstrate a difference in expression indicative of disease. A score was assigned to each test subject on the basis of this determination, where the score of 0 indicates the test subject does not demonstrate a difference of expression indicative of disease (0) and a score of 1 indicates the test subject demonstrates a difference indicative of disease. The total number of correctly identified control samples (ie the TN or true negative fraction) and the total number of correctly identified diseased samples (ie the

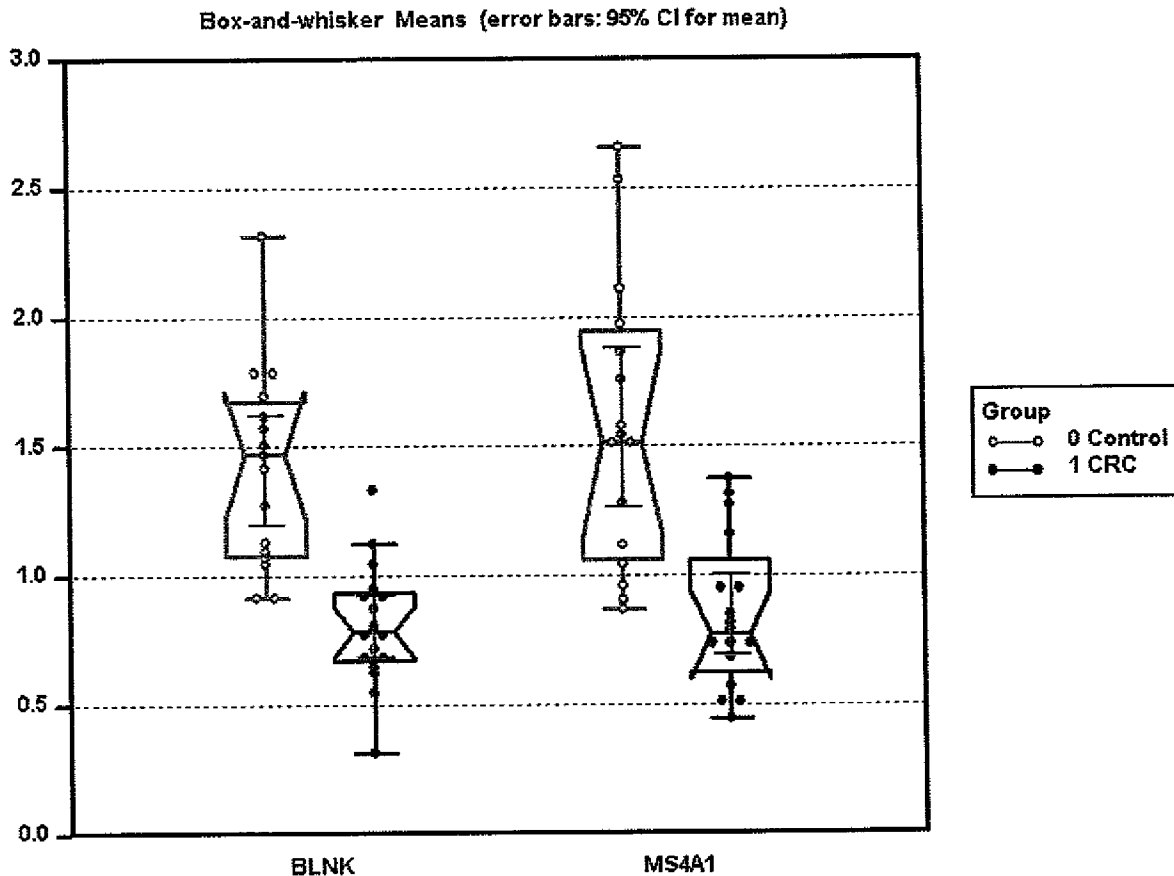
TP or true positive fraction) are noted. From this, the Specificity and Sensitivity of each gene in detecting a difference indicative of disease is noted.

The specificity and sensitivity of the noted method was also determined when the cumulative results determined for each gene was applied to each test individual. Thus, for each disease, the results of each gene (1 or 0) were divided by the total number of genes tested and a cumulative score of greater than 0.5 considered to indicate the subject had disease and a cumulative score of less than 0.5 considered to indicate the subject was a control subject.

Conclusion: Almost all individual genes demonstrated a sensitivity and specificity of greater than 80%. The sensitivity and specificity for detecting diseased increased when using more than two genes.

Example 1. Colorectal cancer (CRC):

- A. Box and Whisker Plot : Total RNA from unfractionated cells of lysed blood from a total of 31 samples from 15 non-CRC (controls) subjects and 16 CRC patients was hybridized to probes of predetermined sequence on the Affymetrix GeneChip U133 Plus 2.0. Patients with CRC included individuals with various levels of colorectal cancer as determined in accordance with Duke staging criteria including 2 individuals stage A, 6 individuals stage B and 8 individuals with stage C colorectal cancer. A set of 51 probe sets each corresponding to a gene ($p < 0.001$, non-parametric, Wilcoxon-Mann-Whitney test) were identified which each demonstrated differential expression as between individuals with CRC patients from that of controls. For purposes of this declaration two genes were selected.



B. Comparing a Test Subject as compared with Control Subjects : A threshold of 1.0 was chosen so as to minimize the number of false positives and false negatives. In most cases the threshold was chosen such that 75 percent of the control subjects were found to be above the threshold. Test subjects demonstrating a quantified level of RNA exceeding the threshold are considered not to demonstrate a difference of expression indicative of disease and assigned a score of 0 indicating a control. Individuals with a quantified level of RNA below the threshold were considered to demonstrate a difference of expression indicative of disease and thus considered to be a subject having colorectal cancer and assigned a score of 1.0. For BLNK1, 13 of the 15 controls were accurately diagnosed as being non diseased and 14 out of 16 diseased subjects were accurately diagnosed as having colorectal cancer resulting in a specificity of 87% and a sensitivity of 82%.

Cumulative Results of Two or More Genes

The results of each of the two genes were averaged (total score / number of genes). Cumulative results of greater than 0.5 were considered to demonstrate the test subject has disease. Cumulative results of less than 0.5 were considered to demonstrate the test subject does not have disease. A score of 0.5 was considered indeterminate (equivocal). 9% of the population was considered equivocal. An overall sensitivity of 71% and a specificity of 80% for the use of two or more genes was found. True controls as

determined by diagnosis are shown in green, true diseased individuals are shown in orange.

			Threshold			
			1.000	1.000		
Sample ID	BLNK Quantified Level RNA	MS4A1 Quantified Level of RNA	BLNK	MS4A1	Avg	Cumulative Results
CTL125	0.916	0.871	1	1	1.00	1
CTL127	0.927	0.905	1	1	1.00	1
CTL174	1.048	1.118	0	0	0.00	0
CTL285	1.071	1.513	0	0	0.00	0
CTL128	1.097	1.040	0	0	0.00	0
CTL204	1.131	1.280	0	0	0.00	0
CTL290	1.275	0.959	0	1	0.50	Indeterminate
CTL192	1.468	1.756	0	0	0.00	0
CTL291	1.506	1.543	0	0	0.00	0
CTL293	1.572	1.972	0	0	0.00	0
CTL287	1.621	1.512	0	0	0.00	0
CTL294	1.698	2.529	0	0	0.00	0
CTL126	1.787	2.106	0	0	0.00	0
CTL295	1.796	1.865	0	0	0.00	0
CTL148	2.317	2.652	0	0	0.00	0
CTL180	0.318	-	1	-	-	Indeterminate
CTL130	0.55	0.446	1	1	1.00	1
CTL176	0.626	0.513	1	1	1.00	1
CTL177	0.651	0.571	1	1	1.00	1
CTL178	0.682	0.814	1	1	1.00	1
CTL131	0.688	0.740	1	1	1.00	1
CTL313	0.722	0.518	1	1	1.00	1
CTL267	0.772	0.738	1	1	1.00	1
CTL101B	0.783	1.275	1	0	0.50	Indeterminate
CTL239	0.800	0.754	1	1	1.00	1
CTL102	0.875	0.790	1	1	1.00	1
CTL129	0.922	0.683	1	1	1.00	1
CTL103	0.930	0.955	1	1	1.00	1
CTL134	0.952	0.960	1	1	1.00	1
CTL171	1.049	1.161	0	0	0.00	0
CTL138	1.123	1.315	0	0	0.00	0
CTL312	1.336	1.373	0	0	0.00	0
			BLNK	MS4A1	Vote	
TN			13	12	12	
TP			14	12	12	
accuracy			84%	77%	75%	
equivocal					9%	
Specificity			87%	80%	80%	
Sensitivity			82%	75%	71%	

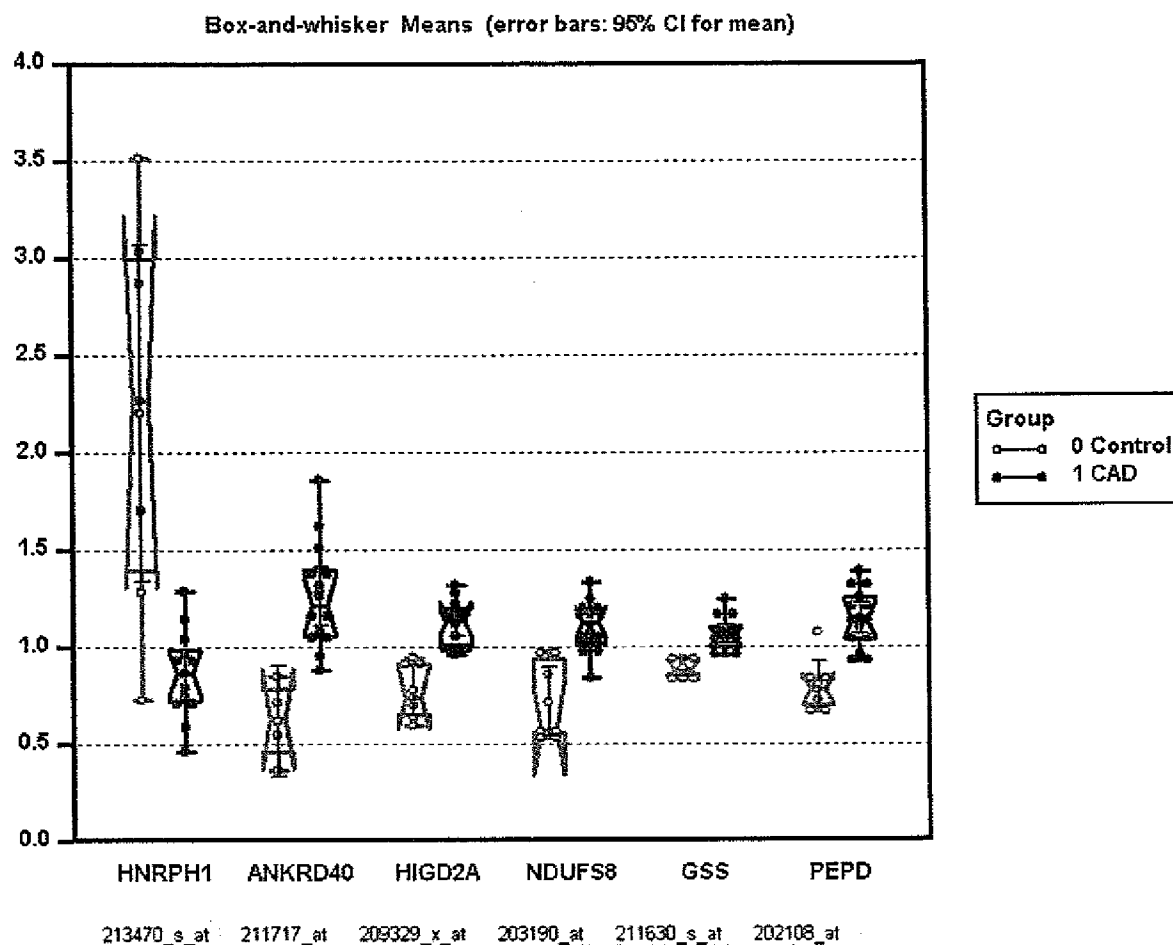
Comparing a Test Subject as compared with a Control Subject : As can be seen in the data presented below, one can compare a test subject with a single control subject, or alternatively, with more than one control, as claimed in the above reference patent application, to determine a difference in gene expression (according to the claim language) which is indicative of disease. One of skill in the art will readily appreciate (as of the instant filing date) that a single control subject may serve as a standard particularly where the chosen control subject is representative of a percentile of a given population of control subjects. In the instant case, control subject CTL134 noted above was chosen as this sample is one which falls within the 75th percentile of the population of control subjects. Test subjects demonstrating a quantified level of RNA for greater than the level demonstrated by control subject CTL134 are considered not to demonstrate a difference of expression indicative of disease and assigned a score of 0 indicating a control. Individuals with a quantified level of RNA below the level demonstrated by control subject CTL134 were considered to demonstrate a difference of expression indicative of disease and thus considered to be a subject having colorectal cancer and assigned a score of 1.0. Results are presented below for both the BLNK and MS4A1 gene. Note that the results do not vary from the previous example comparing the use of a greater number of control samples.

			Threshold			
			1.000	1.000		
Sample ID	BLNK Quantified Level RNA	MS4A1 Quantified Level of RNA	BLNK	MS4A1	Avg	Cumulative Results
CTL125	0.916	0.871	1	1	1.00	1
CTL127	0.927	0.905	1	1	1.00	1
CTL174	1.048	1.118	0	0	0.00	0
CTL285	1.071	1.513	0	0	0.00	0
CTL128	1.097	1.040	0	0	0.00	0
CTL204	1.131	1.280	0	0	0.00	0
CTL290	1.275	0.959	0	1	0.50	Indeterminate
CTL192	1.468	1.756	0	0	0.00	0
CTL291	1.506	1.543	0	0	0.00	0
CTL293	1.572	1.972	0	0	0.00	0
CTL287	1.621	1.512	0	0	0.00	0
CTL294	1.698	2.529	0	0	0.00	0
CTL126	1.787	2.106	0	0	0.00	0
CTL295	1.796	1.865	0	0	0.00	0
CTL148	2.317	2.652	0	0	0.00	0
CTL180	0.318	-	1	-	-	indeterminate
CTL130	0.55	0.446	1	1	1.00	1
CTL176	0.626	0.513	1	1	1.00	1
CTL177	0.651	0.571	1	1	1.00	1
CTL178	0.682	0.814	1	1	1.00	1
CTL131	0.688	0.740	1	1	1.00	1
CTL313	0.722	0.518	1	1	1.00	1
CTL267	0.772	0.738	1	1	1.00	1
CTL101B	0.783	1.275	1	0	0.50	Indeterminate

CTL239	0.800	0.754	1	1	1.00	1
CTL102	0.875	0.790	1	1	1.00	1
CTL129	0.922	0.683	1	1	1.00	1
CTL103	0.930	0.955	1	1	1.00	1
CTL138	0.942	0.960	1	1	1.00	1
CTL171	1.049	1.161	0	0	0.00	0
CTL138	1.123	1.315	0	0	0.00	0
CTL312	1.336	1.373	0	0	0.00	0
			BLNK	MS4A1	Vote	
TN			13	12	12	
TP			14	12	12	
accuracy			84%	77%	75%	
equivocal					9%	
Specificity			87%	80%	80%	
Sensitivity			82%	75%	71%	

Example 2: coronary artery disease (CAD)

A. Box and Whisker Plot : Total RNA from unfractionated cells of lysed blood from a total of 21 samples (including 14 CAD and 7 controls) was hybridized to probes of predetermined sequence on the Affymetrix U133 Plus 2.0 GeneChip. A set of 678 probe sets, each corresponding to a human gene were identified ($p < 0.01$ Wilcoxon-Mann-Whitney non-parametric test) were identified which each demonstrated differential expression as between individuals with the CAD and the control patients. For purposes of this declaration six genes were selected. HNRPH1 in particular is noted on page 36 of US 2004/0014059 as a gene expressed in blood and also expressed in brain, heart, kidney and lung tissue.



B. Comparing Test Subject as compared with Control Subjects : A threshold of 1.3 (HNRPH1), 0.85 (ANKRD40), 0.95 (HIGD2A), 1.0 (NDUFS8), 0.95 (GSS) and 0.85 (PEPD) were chosen such that 75 percent of the control (non diseased) samples were found to be above (or below) the threshold. In the case of ANKRD40, HIGD2A, NDUFS8, GS, PEPD, test subjects demonstrating a quantified level of RNA above this threshold were considered to demonstrate a difference of expression indicative of disease and assigned a score of 1.0 indicating the presence of coronary artery disease. Individuals with a quantified level of RNA below this threshold were considered not to demonstrate a difference of expression indicative of disease and thus considered to be a control subject and assigned a score of 0. In the case of HNRPH1, test subjects demonstrating a quantified level below the threshold of 1.3 were considered to demonstrate a difference of expression indicative of disease and assigned a score of 1.0 indicating the presence of coronary artery disease. Similarly a quantified level of greater than 1.3 indicated no difference in expression indicative of disease and resulted in a score of 1.0. For HNRPH1, a sensitivity of 93% and specificity of 71% resulted for the population. For ANKRD40, a sensitivity of 100%, specificity of 57%; for HIGD2A, a sensitivity of 100% and specificity of 100%; for NDUFS8, a sensitivity of 79% and a

specificity of 100%; for GSS, a sensitivity and specificity of 100% and for PEPD, a sensitivity of 100% and a specificity of 86%.

Cumulative Results of Two or More Genes

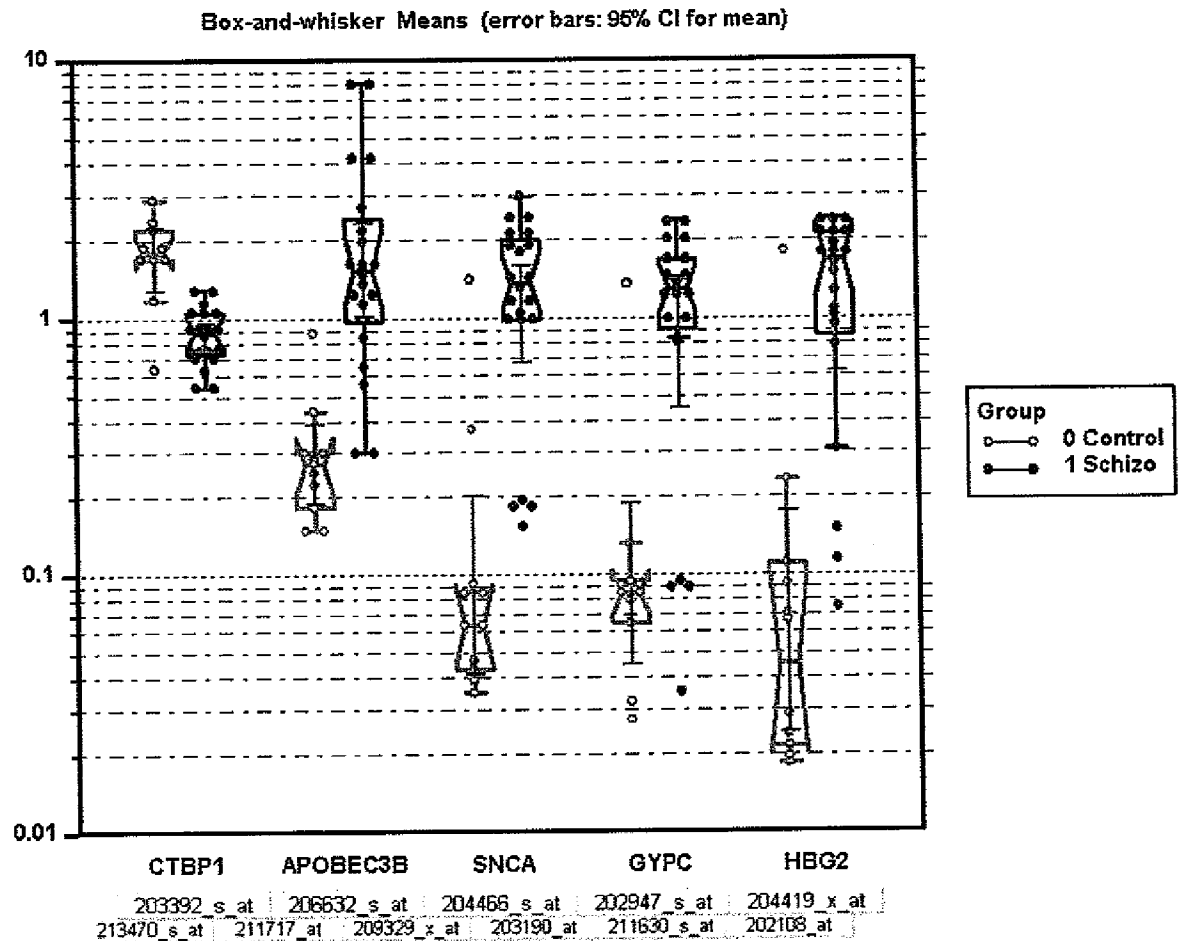
The results of each of the six genes were averaged (total score / number of genes). Cumulative results of greater than 0.5 were considered to demonstrate the test subject has disease. Cumulative results of less than 0.5 were considered to demonstrate the test subject does not have disease. A score of 0.5 was considered indeterminate (equivocal). 9% of the population was considered equivocal. Using the cumulative score 100% sensitivity and specificity resulted. True controls as determined by diagnosis are shown in green, true diseased individuals are shown in orange.

Sample ID	Threshold						Avg	Vote
	1.300	0.850	0.950	1.000	0.950	0.850		
	HNRPH1	ANKRD40	HIGD2A	NDUFS8	GSS	PEPD		
N12B	0	0	0	0	0	0	0.00	0
N48p	0	1	0	0	0	0	0.17	0
N58p	0	1	0	0	0	0	0.17	0
N65A	0	0	0	0	0	0	0.00	0
SU024	1	1	0	0	0	0	0.33	0
SU044	1	0	0	0	0	1	0.33	0
SU050	0	0	0	0	0	0	0.00	0
SU013	1	1	1	1	1	1	1.00	1
SU014	1	1	1	1	1	1	1.00	1
SU020	1	1	1	1	1	1	1.00	1
SU021	1	1	1	0	1	1	0.83	1
SU022	1	1	1	1	1	1	1.00	1
SU040	1	1	1	1	1	1	1.00	1
SU041	1	1	1	1	1	1	1.00	1
SU045	1	1	1	0	1	1	0.83	1
SU046	1	1	1	0	1	1	0.83	1
SU047	1	1	1	1	1	1	1.00	1
SU051	1	1	1	1	1	1	1.00	1
SU052	1	1	1	1	1	1	1.00	1
SU38	1	1	1	1	1	1	1.00	1
SU42	0	1	1	1	1	1	0.83	1
TN TP accuracy equivocal Specificity Sensitivity	ANKRD40							
	HNRPH1	ANKRD40	HIGD2A	NDUFS8	GSS	PEPD		
	(213470_s_at)	(211717_a_t)	(209329_x_at)	(203190_at)	(211630_s_at)	(202108_at)		
	5	4	7	7	7	6	7	
	13	14	14	11	14	14	14	
	86%	86%	100%	86%	100%	95%	100%	
								0%
								100%
								100%

Example 7: Schizophrenia

A. Microarray: Total RNA from unfractionated cells of lysed blood from a total of 30 samples (including 20 schizophrenia subjects and 10 control subjects not having schizophrenia) were hybridized to probes of predetermined sequence on the Affymetrix GeneChip U133 Plus 2.0 GeneChip. A set of 2064 probe sets, each corresponding to a gene ($p < 0.005$, Wilcoxon-Mann-Whitney non-parametric test) were identified which each were differentially expressed as between individuals having schizophrenia and individuals not having schizophrenia.

For the purposes of this declaration five genes were selected. CTBP1 in particular is noted on page 21 of US 2004/0014059 as a gene expressed in blood and also in brain tissue, heart tissue and kidney tissue.



Comparing a Test Subject as compared with Control Subjects : A threshold of 1.15 (CTBP1), 0.45 (APOBEC3B), 0.1 (SNCA), 0.15 (GYPC), 0.1 (HBG2) were chosen such that 75 percent of the control (non diseased) population were found beyond the threshold. In the case of APOBEC3B, SNCA, GYPC, HBG2, test subjects demonstrating a quantified level of RNA above this threshold were considered to demonstrate a difference of expression indicative of disease and assigned a score of 1.0 indicating the presence of schizophrenia. Individuals with a quantified level of RNA below this threshold were considered not to demonstrate a difference of expression indicative of disease and thus considered to be a control subject and assigned a score of 0. In the case of CTBP1, test subjects demonstrating a quantified level below the threshold were considered to demonstrate a difference of expression indicative of disease and assigned a score of 1.0 indicating the presence of schizophrenia. Similarly a quantified level of greater than the threshold indicated no difference in expression indicative of disease and resulted in a score of 1.0. For CTBP1, a sensitivity of 90% and specificity of 90% resulted for the population. For APOBEC3B, a sensitivity of 90%, specificity of 90%; for SNCA, a sensitivity of 100% and specificity of 80%; for GYPC, a sensitivity of 80% and a specificity of 90%; and for HBG2, a sensitivity of 95% and specificity of 70% was determined.

Cumulative Results of Two or More Genes

The results of each of the five genes were averaged (total score / number of genes). Cumulative results of greater than 0.5 were considered to demonstrate the test subject has disease. Cumulative results of less than 0.5 were considered to demonstrate the test subject does not have disease. A score of 0.5 was considered indeterminate (equivocal). Using the cumulative score 100% sensitivity and 86% specificity resulted. True controls as determined by diagnosis are shown in green, true diseased individuals are shown in orange.

Sample ID	Threshold					Avg	Vote
	1.150	0.450	0.100	0.150	0.100		
	CTBP1	APOBEC3B	SNCA	GYPC	HBG2		
MT003	0	0	1	0	1	0.40	0
MT81	1	0	1	1	1	0.80	1
N12B	0	0	0	0	1	0.20	0
N48p	0	0	0	0	0	0.00	0
N58p	0	0	0	0	0	0.00	0
N65A	0	0	0	0	0	0.00	0
N8C	0	0	0	0	0	0.00	0
SU024	0	0	0	0	0	0.00	0
SU044	0	1	0	0	0	0.20	0
SU050	0	0	0	0	0	0.00	0
SU024	1	1	1	0	1	0.80	1
SU044	1	1	1	0	1	0.80	1
SU050	1	1	1	0	1	0.80	1
MT001	1	1	1	0	0	0.60	1
MT004	1	1	1	1	1	1.00	1
MT007	0	1	1	1	1	0.80	1
MT008	1	1	1	1	1	1.00	1

MT42	1	1	1	1	1	1.00	1
MT45	1	1	1	1	1	1.00	1
MT46	1	1	1	1	1	1.00	1
MT50	0	1	1	1	1	0.80	1
MT53	1	1	1	1	1	1.00	1
MT54	1	1	1	1	1	1.00	1
MT59	1	1	1	1	1	1.00	1
MT63	1	0	1	1	1	0.80	1
MT67	1	1	1	1	1	1.00	1
MT74	1	1	1	1	1	1.00	1
MT75	1	1	1	1	1	1.00	1
MT76	1	1	1	1	1	1.00	1
MT77	1	0	1	1	1	0.80	1
	CTBP1	APOBEC3B	SNCA	GYPC	HBG2		Vote
TN	9	9	8	9	7		6
TP	18	18	20	16	19		14
accuracy	90%	90%	93%	83%	87%		83%
equivocal							0%
Specificity	90%	90%	80%	90%	70%		86%
Sensitivity	90%	90%	100%	80%	95%		100%

The above data supports the working examples and conclusions already stated in the specification and provide additional evidence that the claimed methods of detecting a difference in expression of two or more genes by detecting and quantifying a level of RNA encoded by each gene of an unfractionated sample of lysed blood from a test subject and comparing with a quantified level of control RNA encoded by each gene in RNA of unfractionated samples of lysed blood from one or more control subjects, and detecting a difference for each gene which is indicative of disease have utility. The above data uses one of many statistical analytical methods known in the art as of the filing date of the above referenced patent application.

The above data uses one of just many known statistical analysis methods (e.g. notched box and whisker plots) that were readily available to one skilled in the art as of the filing date of the current specification.

All statements made herein of my own knowledge are true, and all statements made on information and belief are believed to be true, and these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Dr. Choong-Chin Liew.....

Date: 12/Dec/04.....